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Comparison of Lysis Filtration and an Automated Blood Culture System (BACTEC) for Detection, Quantification, and Identification of Odontogenic Bacteremia in Children

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Lysis filtration (LyF) was compared with BACTEC PAEDS PLUS in estimating the prevalence of, and sensitivity for, detection of odontogenic bacteremia. Both real bacteremia and simulated bacteremia (seeded blood or saline samples) were assessed to determine the validity of LyF in estimating bacteremia. The simulated bacteremia was also used to assess the reliability of LyF to estimate intensity of bacteremia in CFU per milliliter of blood. Reference organisms were used to assess the abilities of LyF and BACTEC to isolate known oral streptococci. There was no difference in the number of CFU per milliliter of seeded saline, seeded blood, and drop cultures of the organisms plated directly onto agar. Blood cell volume had a negligible effect on the yield of organisms for simulated bacteremia. When LyF and BACTEC were compared, the time to detection of bacteremia was always significantly shorter for BACTEC. For aerobic cultures, these times were 43.7 and 9.6 h, respectively ($P < 0.01$). For anaerobic cultures, these times were 45.1 and 9.9 h, respectively ($P < 0.01$). These differences occurred as well for bacteremia following the extraction of a single tooth, with LyF and BACTEC aerobic cultures taking 78 and 30.5 h, respectively ($P < 0.0001$). For anaerobic cultures, the times were 90.8 and 45 h, respectively ($P < 0.0004$). A preextraction bacteremia was detected on 2.1% of occasions with BACTEC compared to 31% of occasions with LyF ($P < 0.05$) The use of LyF was an effective and reliable means of estimating the intensity of pre- and postextraction bacteremia. The values were 3.6 and 5.9 CFU/ml, respectively ($P < 0.4729$), and the difference was not statistically significant. In summary, BACTEC is quicker than LyF, but less sensitive. LyF provides additional important information in estimating the intensity of bacteremia.

The role of odontogenic bacteremia in the etiology of bacterial endocarditis (BE) is well established, with oral streptococci (30) comprising between 51 and 63% of the organisms detected (12, 18, 29, 33). The most commonly used method of detecting bacteria in blood is by an automated broth culture, which has the advantage of rapid growth of any cultivable organisms (6). The main disadvantage of the broth culture technique is that it does not enable estimation of the intensity of bacteremia (32). This is considered important, because large inocula are required to induce BE in experimental animals. The 90% infective dose (ID_{90}) for experimental animals is 1×10^6 to 2×10^8 CFU/ml (1, 8, 32). The intensity of bacteremia detected in humans under clinical conditions is only 10 to 100 CFU/ml (5, 7, 22, 27). Even when there is established intravascular infection, such as BE, the intensity of bacteremia is approximately 200 CFU/ml, with values ranging from 16 to 310 (2). Thus an ID_{90} in animals is of the order of 10,000 to 1,000,000 greater in intensity than the bacteremia commonly occurring in humans.

The guidelines for antibiotic prophylaxis of BE take account of the risk to susceptible individuals from dental procedures thought to be hazardous because of the high percentage of prevalence of associated bacteremia (8, 11). This highlights an emerging problem, because recent work has demonstrated that procedures excluded from antibiotic prophylaxis, such as placement of a rubber dam, a procedure used in restorative dentistry (25), cause a bacteremia similar to that following dental extractions (18, 25). It is clear that this recent quantitative evidence conflicts with the current guidelines regarding dental procedures that are recommended for antibiotic prophylaxis in patients with cardiac lesions predisposing them to the risk of developing BE. Other factors, such as perioperative immune modulation, may be a contributory factor (19).

In view of this conflicting information, it has been suggested that the policy on antibiotic prophylaxis for dental procedures be revised (21, 25). It is apparent that quantitative data on the intensity of odontogenic bacteremia may play an important part in assessing the need for antibiotic prophylaxis for specific dentogingival manipulative procedures (25). The availability of such data may lead to a reduction in the number of the specific dental procedures that require antibiotic prophylaxis (9). If this is true, antibiotic use would be reduced. This is an important consideration in view of current anxieties concerning excessive use of antibiotics and the development of antibiotic-resistant organisms (13, 31). Conversely, should the number of procedures requiring prophylaxis be increased, there would be a rational basis to justify the increased use of antibiotics in children. This issue of antibiotic prophylaxis is important, because there is evidence that patients who receive antibiotics for pro-

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phylaxis harbor antibiotic-resistant streptococci in their oral flora (4, 13, 20, 24, 28, 31).

There are no clinical trials on prophylactic antibiotics in the prevention of BE, probably because in excess of 6,000 patients would be required for a robust test (10). In practical terms, this number is unachievable. Current recommendations for dental procedures requiring prophylaxis are based on indirect data from dental bacteremia studies (17). The use of blood culture methods for estimating the intensity of bacteremia, as well as the percentage prevalence of positive cultures, would improve the data available to national bodies when considering dental procedures that should be carried out under the protection of systemic antibiotic prophylaxis.

Preliminary work has shown that the technique of lysis filtration (LyF) appears to be an effective method of estimating the intensity of bacteremia following dental extractions in adults (14, 15). There are no data available on the use of LyF with children.

The purpose of the present work was to investigate the reliability of LyF in the detection of dental bacteremia by comparing it with the reliability of an automated blood culture system, the BACTEC 9240.

MATERIALS AND METHODS

Ethical approval was obtained from The Eastman Dental Hospital Research and Ethics Committee and The Great Ormond Street Hospital for Children NHS Trust.

Effect of blood composition and processing procedures on prevalence and intensity of simulated bacteremia. Blood was obtained by using an aseptic technique from children receiving comprehensive dental treatment under general anesthesia at The Eastman Dental Hospital. The blood was sampled immediately after anesthetic induction and before any dental treatment was carried out. The first 0.5 ml of blood was discarded to void any skin contaminants. This is not normally recommended (26). However, it has been shown that 10% of these "discards" contain staphylococci, probably picked up as a needle traverses a hair follicle (G. J. Roberts, unpublished M.Phil. thesis). A 14-ml sample of blood was withdrawn and divided into two aliquots of 1 ml each as well as two further aliquots of 6 ml each. One milliliter of blood was used for a standard laboratory leukocyte count and hemoglobin and hematocrit estimates. The first 6-ml aliquot of blood was added to 1.23 ml of sodium polyanetholesulfonate to prevent clotting. This 6 ml of blood was seeded with 100 μl (giving approximately 100 CFU) of *Streptococcus sanguis* type strain NCTC 7863 prepared from an overnight broth culture. This was processed by LyF (15). Briefly, each blood sample was added to lysing solution and incubated at 37°C for 10 min. The lysed blood was drawn through a 0.45-μm-pore filter by negative pressure of approximately 60 mm Hg. The filter was removed from the unit and cut in half with sterile scissors. Each half-filter was placed onto brain heart infusion (BHI) agar (Difco, United Kingdom). One plate was incubated aerobically and the other was incubated anaerobically for 10 days. Colonies growing on the filter were subjected to Gram staining and identified to the species level by standard laboratory methods (3). The second 6-ml aliquot of blood was processed by LyF without any added bacteria. Control samples of bacteria suspended in saline were prepared as follows. Ten 12-ml samples of sterile aqueous 0.9% (wt/vol) sodium chloride were prepared, and each was seeded with 200 μl of *S. sanguis.* The seeded saline was divided into two aliquots of 6 ml each giving 100 μl per 6-ml sample. The first aliquot was added to streptokinase-streptodornase lysing solution and processed by LyF. The other 6-ml aliquot was processed through the micropore filter without the streptokinase-streptodornase lysing constituents. (20) In addition, two single 50-μl volumes of seeded saline, which between them contained approximately 100 CFU/ml, were inoculated onto BHI agar as "drop plates" for aerobic and anaerobic incubation, respectively. This provided a method of direct estimation of the number of CFU per 100 μl of the seeding solution used for LyF.

Simulated quantitative blood cultures for BACTEC and LyF with blood seeded with reference microorganisms. The subject volunteers comprised 50 healthy adults at the Eastman Dental Institute and Hospital. By an aseptic technique, 18 ml of blood was withdrawn from a vein in the antecubital fossa. This was collected in a Vacutainer tube containing heparin to prevent clotting. Each blood sample was divided into two unequal aliquots of 6 and 12 ml. The order of processing was determined with random number tables. The 6 ml was equally divided between aerobic and anaerobic BACTEC PAEDS PLUS bottles (Becton Dickinson UK, Ltd., Oxford, United Kingdom). These were processed with the BACTEC 9240 at The Great Ormond Street Hospital for Children, London, United Kingdom. The 12-ml aliquot was seeded with 100 μl of one of several type strains of oral streptococci. A run of 10 blood samples was carried out for each of the five *Streptococcus* type strains: *S. mutans* NCTC 10449, *S. mitis* NCTC 551, *S. intermedius* NCDO 2227, *S. oralis* NCTC 11427, and *S. sanguis* NCTC 7863. Each species was prepared as for the simulated blood cultures used for the hemoglobin study described above. This seeded aliquot of 12 ml was divided into two 6-ml aliquots. The first was divided between aerobic and anaerobic BACTEC PAEDS PLUS bottles and processed in the same way as the unseeded aliquots. The second 6-ml aliquot of the seeded aliquot was processed by LyF as described above. Bacteria isolated from both BACTEC and LyF were reidentified to check that they were the seeded species used and to identify contaminants.

The BACTEC screens for positive cultures automatically every few hours. The filters for LyF were screened twice daily at 0900 and 1400 h with a stereo microscope.

In addition, the effects of the processing on the yield of organisms were examined by comparing the simulated blood culture with saline seeded with identical reference organisms. These results were expressed as CFU per milliliter of seeded blood or seeded saline. These were compared with the yield of reference organisms from a "drop" culture of the broth onto blood agar. This represents 100 μl of the broth of *S. sanguis* type strain NCTC 7863, which gave approximately 100 CFU per plate.

The results were expressed as (i) the time to detection of the bacteremia for both the BACTEC and LyF methods, (ii) the percentage yield from the simulated blood cultures of the five reference organisms for both the BACTEC and the LyF, and (iii) the intensity of bacteremia for the seeded cultures and the seeded saline expressed in CFU per milliliter.

Detection of bacteremia following extraction of a single tooth. The subjects were children at The Great Ormond Street Hospital For Children receiving comprehensive dental treatment under general anesthesia. Immediately upon administration of anesthesia and before dental treatment was started, a 23-gauge intravenous cannula was inserted into a vein of the antecubital fossa or the dorsum of the hand. A 12-ml sample of blood was withdrawn before any dental treatment was started (preprocedure sample). A single dental extraction was performed before any other dental treatment. The second 12-ml blood sample was taken 30 s after the maximum movement involved in a single tooth extraction (23). This was the postextraction sample.

Each 12-ml sample was divided into two 6-ml aliquots. The first was divided into two 3-ml volumes that were inoculated into the BACTEC PAEDS PLUS aerobic and anaerobic bottles, respectively. These were then processed in the BACTEC 9240. The second aliquot was processed by LyF (described above).

The results were expressed (22) in the same way as those for the simulated bacteremia.

Contamination was assessed by processing a pair of BACTEC bottles with every 10th run of samples. The estimation of contamination for the processing of LyF samples was achieved by processing one filter, which was removed from the sterile envelope and put straight onto an agar plate. This was carried out for each set of samples processed.

Statistical methods. Numerical data were tested for normality by using the Shapiro-Wilk test (STATA reference manual, release 3. 5 ed., Computing Resource Center, Santa Monica, Calif.) and found to be not normally distributed. Thus the Mann-Whitney U test was used to test differences between the groups studied. Percent prevalence data were subjected to the chi-square test. Associations and correlations were examined by using the Spearman correlation coefficient.

RESULTS

Simulated blood cultures and the effect of human blood composition and processing procedures on the prevalence and intensity of the simulated bacteremia. The number of CFU per milliliter estimated from the runs of the blood- and saline-simulated bacteremia are shown in Table 1. This demonstrated that for both aerobic and aerobic culture methods, there was no difference between total counts from the saline-simulated

TABLE 1. Number of CFU per 3-ml sample for simulated bacteremia (by seeding blood), simulated saline bacteremia (by seeding saline), and drop cultures

Sample (n = 10)	No. of CFU/3-ml sample				
	Mean[a]	SD	Median	Maximum	Minimum
Aerobic					
Seeded blood (3 ml)	38.7 (*a*)	10.0	40.5	18.5	66.0
Seeded saline (3 ml)	37.8 (*c*)	12.0	38.7	18.0	85.0
Anaerobic					
Seeded blood (3 ml)	38.4 (*b*)	10.6	37.0	15.0	66.0
Seeded saline (3 ml)	36.0 (*d*)	12.7	37.0	15.0	92.0
Drop culture (100 μl)	74.9 (*e*)	25.5	69	55	135

[a] $a + b$ should equal e (38.7 + 38.4 = 77.1; cf 74.9) (not significantly different), and $c + d$ should equal e (37.8 + 36.0 = 73.8; cf 74.9) (not significantly different).

bacteremia and the drop culture. Similarly for the blood-simulated bacteremia, there was no difference between the total count obtained from blood compared with the count from the drop culture.

Simulated blood cultures for BACTEC and LyF with blood seeded with reference microorganisms. As a result of pilot work, there was the suggestion that there was a slightly lower yield of oral streptococci in the LyF. This made it necessary to explore the possibility that LyF was less reliable at detecting oral streptococci than BACTEC. A total of 50 subject volunteers provided blood sampled from the antecubital fossa by an aseptic technique. For each of 10 blood samples selected at random, a type strain was inoculated into the blood and gently mixed. The samples were processed (24) as for the simulated bacteremia (described above). The time to detection was always statistically significantly less for the BACTEC than for LyF (Table 2).

Oral streptococci were recovered from all of the blood samples inoculated into aerobic and anaerobic BACTEC PAEDS plus seeded with type strains *S. mutans* (n = 10), *S. intermedius* (n = 10), *S. oralis* (n = 10), and *S. sanguis* (n = 10). *S. mitis* (n = 10) was recovered from eight of the aerobic bottles and nine of the anaerobic bottles (Table 3). For LyF, the seeded strains were also recovered from all of the samples, except for blood seeded with *S. mitis.* There was no aerobic or anaerobic growth from four samples (Table 3). The difference between BACTEC and LyF was not significant (Fisher's exact probability test P = 0.155). The type strains were reidentified with the API Strep 20 system. There was one positive aerobic culture only in the unseeded group, which was an *Actinobacillus* species, presumably due to contamination. Overall, the prevalence of contamination was less than 1%.

Detection of bacteremia following extraction of a single tooth. The patients were 49 children 2 years 11 months to 14 years 11 months of age, with a mean age of 8 years 3 months. The percent prevalence values of bacteremia both preextraction and 30 s after extraction of a single tooth were greater with LyF (Table 4). This was statistically significantly different for the preextraction samples ($P < 0.05$), but not for the postextraction samples. The intensity of bacteremia preextraction was 3.6 (standard deviation [SD], 6.2), and the intensity postextraction was 5.9 (SD, 8.2), a difference that was not statistically significantly different (P = 0.4729) (Table 5). The mean time for detection of positive cultures was statistically significantly shorter ($P < 0.0004$) for BACTEC, with both aerobic and anaerobic methods requiring less than half the time taken for LyF (Table 6). A wide variety of bacteria were isolated with both BACTEC and LyF. These included staphylococci, streptococci, micrococci, and neisseria and corynebacteria (Table 7). There were slightly more oral streptococci isolated with BACTEC than with LyF: 31.2 and 17.6%, respectively. This was not statistically significantly different (chi square = 1.661, degree of freedom = 1, P = 0.197).

DISCUSSION

The effect of human blood of the lysing solution on the yield of organisms for LyF under both aerobic and anaerobic con-

TABLE 2. Time to detection of simulated bacteremia using seeded type strains for BACTEC versus LyF

Method (n = 50)	No. of h to detection[a]				
	Mean	SD	Median	Min	Max
Aerobic					
BACTEC	9.6	5.3	8.0	5.2	22.8
LyF	43.7	20.8	48.0	24.0	96.0
Anaerobic					
BACTEC	9.9	5.7	8.4	5.8	25.4
LyF	45.1	20.2	48.0	24.0	96.0

[a] BACTEC had a statistically significantly shorter time to detection than LyF for aerobic ($P < 0.01$) and anaerobic ($P < 0.01$) conditions.

TABLE 3. Number of positive cultures from simulated blood cultures (oral streptococci) detected by BACTEC and LyF

Species of type strain (n = 10)[b]	No. of positive cultures[a]			
	BACTEC		LyF	
	Aerobic	Anaerobic	Aerobic	Anaerobic
S. mutans	10	10	10	10
S. mitis	8	9	6	6
S. intermedius	10	10	10	10
S. oralis	10	10	10	10
S. sanguis	10	10	10	10

[a] No statistically significant differences between strains for detection prevalence were detected.

[b] n = 10 for each strain.

TABLE 4. Prevalence of positive cultures for BACTEC versus LyF ($n = 49$)

Bacteremia period	% Prevalence		Significance[a]
	BACTEC	LyF	
Preextraction	2.1	31.0	$P < 0.05$ (significant)
Postextraction	34.7	42.9	$P > 0.05$ (not significant)

[a] Significant difference between BACTEC and LyF determined for preprocedure (baseline) only.

ditions for the simulated bacteremia was negligible. Neither the natural blood antibacterial factors nor the enzymes used in the lysing process had any effect on the viable count. The differences between the seeded blood and seeded saline confirmed this, with values that are remarkably close. This is reassuring, because there is the possibility that the enzymes used for the lysing process could affect the viability of oral streptococci. In addition, the total sample counts in CFU per milliliter were identical to the counts on "drop" cultures of the same specially prepared broth of reference organisms for both the blood samples and the saline samples. This is clearly an advantage of LyF, because it enables investigators to reliably estimate the intensity of bacteremia. The similarity of CFU counts from the simulated bacteremia, saline bacteremia, and drop cultures gives considerable support to this claim. This is an advantage over the BACTEC, because broth cultures do not enable estimation of the intensity of bacteremia.

An advantage of the broth culture is the very rapid response: a simulated bacteremia was detected within 10 h, compared to over 40 h with LyF. This is important clinically, because it enables BACTEC to detect a potentially life-threatening septicemia very rapidly. The importance of this finding is that lysis filtration is not a substitute for BACTEC broth cultures for the detection of clinically important bacteremia.

The abilities of both BACTEC and LyF to detect different reference organisms in simulated bacteremia were extremely good. For four out of five species, this detection was 100%. Both systems were less good at detecting *S. mitis.* This is perplexing, and it is not possible to offer an explanation for this other than the possibility that *S. mitis* is, in general, less easy to culture reliably.

The prevalence of bacteremia detected in child patients was greater with LyF both pre- and postextraction of a tooth compared with the broth culture There is no obvious explanation for the greater recovery of bacteria from the preoperative LyF compared with the preoperative BACTEC. It is possible that with low levels of bacteria, the BHI medium is more effective than broth at encouraging growth. This is in agreement with earlier work (16) and could also be of clinical significance for the prescription of antibacterial drugs, particularly if the broth culture is negative. A preoperative sample is recommended to give a realistic estimate of postoperative bacteremia. This is because some preextraction bacteremia may be as high as 20 CFU/ml. It would be necessary to have a sample size of 157 subjects to achieve statistical significance (STATA reference manual, release 3.5 ed.).

TABLE 5. Intensity of bacteremia by LyF[a]

Bacteremia period (n)	Bacteremia intensity (CFU/ml)				
	Mean	SD	Median	Minimum	Maximum
Preprocedure (15)	3.6	6.2	2	1	20
Postextraction (21)	5.9	8.2	2	1	28

[a] Note that only positive cultures were used for these calculations. If the mean value for all 49 patients in the group is computed, then the mean for preprocedure bacteremia is 0.67 CFU/ml and that for postprocedure bacteremia is 1.92 CFU/ml of blood. $P < 0.4729$ (not significant).

TABLE 6. Time in hours to detection of positive blood cultures following a single dental extraction for BACTEC versus LyF in 49 children[a]

Sample (n)	Time to detection (h)					Significance
	Mean	SD	Median	Minimum	Maximum	
Aerobic						$P < 0.0001$
BACTEC (17)	30.5	11.2	24	24	48	
LyF (21)	78.0	74.1	48	24	264	
Anaerobic						$P < 0.0004$
BACTEC (17)	45.0	20.0	48	24	72	
LyF (21)	90.8	84.7	48	24	264	

[a] A total of 49 children provided a 3-ml sample for aerobic and aerobic processing for BACTEC and LyF: hence $49 \times 4 = 196$ blood samples were processed.

The reliability and consistency of BACTEC for the detection of odontogenic bacteremia have been confirmed, because the percentage of positive cultures detected in this study (35.7%) was similar to that in a previous study conducted by the same investigators (43.2%) by a radiometric method for identification of positive cultures (24).

The technique of LyF used in this study on children was not as sensitive as that in a study of adults, in which 86% of blood cultures were positive following a single dental extraction (16). This may be because the subjects in the present study were children, and the percentage of prevalence-positive cultures

TABLE 7. Bacterial species isolated from positive blood cultures following extraction of a single tooth

Bacteria	No. of isolates from culture			
	Aerobic		Anaerobic	
	BACTEC	LyF	BACTEC	LyF
Staphylococcus aureus			1	
Staphylococcus epidermidis	4	3	6	
Staphylococcus capitis			1	
Streptococcus mitis	1	2	1	2
Streptococcus sanguis	2	3	1	1
Streptococcus gordonii	1		1	
Streptococcus salivarius	1			
Streptococcus porcinus	2			
Enterococcus faecalis	2		4	
Micrococcus sp.	2	1	1	
Bacillus megaterium	1		1	
Bacillus licheniformis	1		1	
Bacillus pumilus			1	1
Aerococcus urinae			1	
Veillonella sp.			1	3
Neisseria sp.		2		
Corynebacterium sp.	2		2	1
Diphtheroids		1		
Gram-positive rods		1	3	

following dental treatment in children is almost always less than that in adults (32). What is clear is that the use of LyF is more sensitive than BACTEC for the detection of the percentage of prevalence of bacteremia following the extraction of a single tooth.

The ability of LyF to estimate the intensity of bacteremia was confirmed with values ranging from 1 to 28 CFU/ml following the extraction of a single tooth. This compares well with the data for intensity in adults, for which the values were up to 43 CFU/ml (16), a mean of 3.8 CFU/ml (14), and a mean of 21 CFU/ml (17), respectively.

As with the simulated bacteremia, the time to detection of the clinical bacteremia was very much greater with the LyF (29) (Table 6). This confirms the superiority of BACTEC in detecting odontogenic bacteremia in patients in terms of the rapidity of obtaining a result.

The range of organisms isolated following the single dental extraction appears greater with BACTEC than with LyF and probably reflects the ability of BACTEC to detect very low levels of some bacteremia. This is an issue that should be investigated further. In perusing the data, BACTEC appears to be better at detecting staphylococci and some streptococci.

In conclusion, LyF is significantly more sensitive than BACTEC at detecting odontogenic bacteremia and has the ability to detect almost the same range of organisms. It has the disadvantage that it is very much slower than BACTEC and, at present, is limited to research. The ability of LyF to estimate the intensity of bacteremia may be important in monitoring the progress of BE once a diagnosis has been made.

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